

Application No. 10/054,728
Filed October 25, 2001
Page 2 of 17

AMENDMENTS TO THE CLAIMS

1. (currently amended) A biochip comprising:
- a) a solid substrate having a surface;
 - b) ~~at least one~~ a plurality of optically clear three-dimensional hydrogel ~~cell~~ cells attached to the surface of the substrate at discrete locations in an array, which hydrogel ~~cell~~ cells are formed from an isocyanate-functional ~~polymer~~ prepolymer; and
 - c) a different binding entity immobilized within or upon various of said hydrogel ~~cell~~ cells, which entity is effective to selectively hybridize to or sequester a target molecule.
2. (original) The biochip of claim 1 wherein the hydrogel comprises a polymer with urethane linkages.
3. (currently amended) The biochip of claim 1 wherein the hydrogel comprises polyethylene glycol, polypropylene glycol, or copolymers thereof having a molecular weight of at least about 5000.
4. (original) The biochip of claim 1 wherein the hydrogel cell is at least 20 μm thick.
5. (original) The biochip according to claim 4, wherein the hydrogel cell is between about 30 μm and about 100 μm thick.

BEST AVAILABLE COPY

Application No. 10/054,728
Filed October 25, 2001
Page 3 of 17

6. (original) The biochip according to claim 1, wherein said binding entity is covalently bound to and within the hydrogel cell through reaction with isocyanate groups.

7. (previously presented) The biochip of claim 6 wherein about 15% or less of the reactive isocyanates in said polymer of said cell have reacted with said binding entities.

8. (withdrawn) The biochip of claim 1 wherein with each said binding entity comprises DNA, RNA or PNA.

9. (currently amended) The biochip of claim 1 wherein each said binding entity comprises an immunoglobulin, an enzyme, a receptor, an enzyme inhibitor, an enzyme substrate, or a peptide.

10. (original) The biochip of claim 9 wherein each said binding entity is immobilized within the hydrogel through an interaction with an intermediate agent.

11. (withdrawn) The biochip of claim 1 wherein each said binding entity is a protein which is bound to a metal chelate that is immobilized in the hydrogel and constitutes said intermediate agent.

BEST AVAILABLE COPY

Application No. 10/054,728
Filed October 25, 2001
Page 4 of 17

12. (withdrawn) The biochip of claim 11 wherein said protein is bound to said metal chelate through a histidine-containing polypeptide at one terminal of said protein.

13. (withdrawn) The biochip of claim 1 wherein each said binding entity is immobilized through a first intermediate agent linked to the hydrogel and a second intermediate agent linked to said first intermediate agent.

14. (withdrawn) The biochip of claim 13 wherein said first intermediate agent is an antibody and the second intermediate agent is a protein.

15. (cancelled)

16. (original) The biochip of claim 5 wherein said surface has a plurality of wells formed therein.

17. (currently amended) The biochip of claim 1 wherein the substrate is optically transparent and has reactive molecules on its top surface to which the hydrogel is covalently bound through some of said isocyanate groups of the polymer.

18. (currently amended) A hydrogel biochip comprising:

a) a solid substrate having a top surface;

BEST AVAILABLE COPY

Application No. 10/054,728
Filed October 25, 2001
Page 5 of 17

- b) a plurality of optically clear three-dimensional hydrogel cells comprising polyethylene glycol, polypropylene glycol, or copolymers thereof bound to the top surface of said substrate at discrete locations in an array;
- c) intermediate agents immobilized within or upon ~~said hydrogel~~ of said hydrogel cells; and
- d) different protein binding entities bound to said intermediate agents respectively within at least several of said hydrogel cells by interaction therewith in a manner so that said protein binding entities assume their native conformations.

19-30. (cancelled)

31. (currently amended) A biochip comprising:

- a) a solid substrate having a surface;
- b) ~~at least one~~ a plurality of optically clear three-dimensional hydrogel ~~cell~~ cells attached to the surface of the substrate at discrete locations in an array, ~~which each~~ hydrogel cell ~~is being~~ formed from an isocyanate-functional polymer; and
- c) a different protein binding entities immobilized within or upon different said hydrogel ~~cell~~ cells, ~~which each~~ protein binding entity is being effective to selectively hybridize to or sequester a target molecule.

32. (currently amended) The biochip of claim 31 wherein the hydrogel comprises polyethylene glycol, polypropylene glycol, or copolymers thereof having a molecular weight of at least about 5000 with urethane linkages to polyisocyanates.

BEST AVAILABLE COPY

Application No. 10/054,728
Filed October 25, 2001
Page 6 of 17

33. (currently amended) The biochip of claim 32 wherein ~~the~~ each hydrogel cell is between about 20 μm and about 100 μm thick.

34. (currently amended) The biochip of claim 31 wherein each said protein binding entity is covalently bound to and within the hydrogel cell through reaction with isocyanate groups.

35. (currently amended) The biochip of claim 31 wherein with said protein ~~comprises an immunoglobulin~~ binding entities comprise immunoglobins.

36. (withdrawn) The biochip of claim 31 wherein each said protein binding entity comprises an enzyme, a receptor, an enzyme inhibitor or an enzyme substrate.

37. (cancelled)

38. (withdrawn) The biochip of claim 31 wherein each said protein binding entity is immobilized through a first intermediate agent that is covalently bound to isocyanate groups in the hydrogel and a second intermediate agent linked to said first intermediate agent.

39. (withdrawn) The biochip of claim 38 wherein said first intermediate agent is an antibody and said second intermediate agent is a protein.

BEST AVAILABLE COPY

Application No. 10/054,728
Filed October 25, 2001
Page 7 of 17

40. (withdrawn) The biochip of claim 38 wherein said intermediate agents comprise biotin and avidin.

41. (currently amended) A biochip comprising:

- a) a solid substrate having a top surface;
- b) a plurality of optically clear three-dimensional hydrogel cells comprising polymers of (i) polyethylene glycol, polypropylene glycol, or copolymers thereof and (ii) polyisocyanates, which cells are bound to the top surface of said substrate at discrete locations in an array;
- c) intermediate agents immobilized within or upon said ~~hydrogel~~ of said hydrogel cells; and
- d) different protein binding entities bound to said intermediate agents within at least several of said hydrogel cells by interaction therewith in a manner so that said protein binding entities can assume their native conformations.

42. (previously presented) The biochip of claim 41 wherein said hydrogel is a urethane polymer formed from a prepolymer with excess isocyanate groups, and wherein said protein binding entities are bound through pairs of intermediate coupling agents.

43. (new) The biochip of claim 41 wherein said intermediate agent is nitrilotriacetic acid.

BEST AVAILABLE COPY

Application No. 10/054,728
Filed October 25, 2001
Page 8 of 17

44. (withdrawn) A method of carrying out a biochemical assay, which method comprises the steps of:

- (a) providing a biochip according to claim 31 having a plurality of optically clear hydrogel cells bound to a surface of a substrate,
- (b) contacting said hydrogel biochip with an analyte solution potentially containing target biomolecules under binding conditions;
- (c) washing said hydrogel biochip under conditions that remove non-selectively bound and unbound biomolecules; and
- (d) detecting target biomolecules bound to any one of said cells.

45. (withdrawn) The method of claim 44 wherein binding of a target biomolecule results in a compositional change of the protein binding entity in the form of a phosphorylation event or dephosphorylation event.

BEST AVAILABLE COPY